Dietary Docosahexaenoic Acid and Immunocompetence in Young Healthy Men

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ABSTRACT: The purpose of this study was to examine the effect of dietary docosahexaenoic acid (DHA), in the absence of eicosapentaenoic acid, on human immune response (IR). A 120-d study with 11 healthy men was conducted at the Metabolic Research Unit of the Western Human Nutrition Research Center. Four subjects (control group) were fed the stabilization or basal diet (15, 30, and 55% energy from protein, fat, and carbohydrate, respectively) throughout the study; the remaining seven subjects (DHA group) were fed the basal diet for the first 30 d, followed by 6 g DHA/d for the next 90 d. DHA replaced an equivalent amount of linoleic acid; the two diets were comparable in their total fat and all other nutrients. Both diets were supplemented with 20 mg d-α-tocopherol acetate per day. Indices of IR were examined on study day 22, 30, 78, 85, 106, and 113. Addition of DHA at moderately high levels did not alter the proliferation of peripheral blood mononuclear cells cultured with phytohemagglutinin or concanavalin A, or the delayed hypersensitivity skin response. Also, additional DHA did not alter the number of T cells producing interleukin 2 (IL2), the ratio between the helper/suppressor T cells in circulation, or the serum concentrations of immunoglobulin G, C3, and interleukin 2 receptor (IL2R). DHA supplementation, however, caused a significant ($P = 0.0001$) decrease in the number of circulating white blood cells which was mainly due to a decrease in the number of circulating granulocytes. The number of lymphocytes in peripheral circulation was not affected by Dietary DHA enrichment, but the percentage of lymphocytes in white blood cells increased because of a reduction in granulocyte numbers. None of these indices was changed in the control group. Our results show that when total fat intake is low and held constant, DHA consumption does not inhibit many of the lymphocyte functions which have been reported to be inhibited by fish oil consumption.
Lipids 33, 559–566 (1998).

Both the amount and type of dietary fat modulate human immune response (IR). A reduction in total fat intake enhances several indices of IR, and the converse occurs with an increase in dietary fat (1–4). A moderate increase in the consumption of the n-6 polyunsaturated fatty acids (PUFA), linoleic acid (LA), or arachidonic acid did not lower the human IR when total fat intake was maintained constant (1,2,5). But n-6 PUFA lowered human IR when its addition to the diet caused an increase in the total fat intake (4). The addition of n-3 PUFA from fish oil or flaxseed oil also reduced several aspects of the human IR (6–19). Since total fat intake was not kept constant in most of these studies, it is difficult to determine if the decline in IR was due to the amount or the type of fat, or a combination of the two factors. Because fish oils cause a decrease in IR, their use has been explored in the management of autoimmune disorders (20–24).

Fish oils contain a variable mixture of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), along with many other fatty acids, cholesterol, heavy metals, and chlorinated hydrocarbons. It is not known if the decline in IR caused by the fish oils is due to EPA, DHA, both, or some other factors. Purified esters of EPA lowered several aspects of the IR in humans (13,14,19). *In vitro* studies conducted with human (25) and rat lymphocytes (26) and *in vivo* studies in rats (27) showed that DHA esters/salts also inhibit immune cell functions. Until recently, purified DHA was not available for human use, and no studies regarding its effects on human IR have been done. Studies with DHA are important, because it is the major n-3 fatty acid in tissues, and the body tends to conserve it over EPA. Such studies are now possible with the availability of DHASCO^{TM} oil (a gift from Martex Corporation, Columbia, MD), which contains 40% DHA as natural triacylglycerol.

The purpose of this study was to examine the effect of a moderately high intake (6 g/d) of DHA (in the absence of EPA) on human immunocompetence, while dietary fat was held constant. To avoid increased oxidative stress from the consumption of DHA, 20 mg d-α-tocopherol acetate was added to the diets daily. We examined the effect of feeding DHASCO^{TM} oil (15 g/d) for 83 d on several indices of immune status [complete and differential blood cell count, lymphocyte phenotypic analysis and *in vitro* proliferation, serum immunoglobulin G (IgG), C3, and interleukin 2 receptor (IL2R)] in healthy men. In previous studies with dietary fish oil these indices were found to be altered.

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Abbreviations: Bq, Becquerel; CD, cluster designation; Con A, concanavalin A; CTL, cytotoxic T; DHA, docosahexaenoic acid; DTH, delayed type hypersensitivity skin response; EPA, eicosapentaenoic acid; IL, interleukin; IR, immune response; LA, linoleic acid; LTB_4, leukotriene B_4; PBMNC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PMN, polymorphonuclear leukocytes; PUFA, polyunsaturated fatty acid; WBC, white blood cell.

MATERIALS AND METHODS

Subjects and study design. The study protocol was approved by the Human use committees of the University of California at Davis, and the USDA committee (Houston, TX). Twelve healthy men were selected for the study after a physical and clinical examination by a licenced physician. The study lasted for 120 d (April 1 to July 29, 1996) although no immunological tests were conducted after d 113, because of other scheduled procedures. Subjects lived at the Metabolic Research Unit of the Western Human Nutrition Research Center for the duration of the study, except when going for daily walks (2 × 2 miles) or other scheduled outings. They consumed only those foods prepared by the staff of the Metabolic Research Unit, and were under constant supervision. Subjects were divided into two groups: a control group of four was fed the stabilization or basal diet for all 120 d of the study; the remaining eight subjects (DHA group) were fed the basal diet for the first 30 d of the study and a DHA-supplemented diet for the last 90 d. One subject from the DHA group did not complete the study; hence, data from only seven subjects were evaluated to determine the effects of DHA on IR.

Both diets were intended to provide 15, 30, and 55% energy from protein, fat, and carbohydrates, respectively. As shown in Table 1, the analyzed compositions of each of the two diets were not different from one another or from the planned values. Diets composed of natural foods, except DHA and vitamin E, were fed in a 5-d rotating menu, comprising three meals and a post-dinner snack. Body weights of the subjects were maintained within 2% of their initial weights throughout the study, by adjusting their energy intake if necessary. For the DHA-supplemented group, 15 g of DHASCO^{TM} oil was incorporated by replacing an equivalent amount of safflower oil; all other foods were identical between the two diets. The DHASCO^{TM} oil, purified from an alga, contains 40% DHA as triacylglycerol. It also contains 12:0 (3.8%), 14:0 (13.5), 16:0 (11.5%), 18:0 (1.2%), 18:1n-9 (27.9), and the balance as minor components. The main effect of incorporating 15 g DHASCO^{TM} oil by replacing an equivalent amount of safflower oil was to replace 6 g linoleic acid (LA) with the same amount of DHA. The DHASCO^{TM} oil was kept in sealed containers at –20°C, and all open bottles were flushed with nitrogen before returning to the refrigerator. It was served only in cold foods such as yogurt, dips, or salads. Thus, the chances of DHA oxidation were minimized. Dietary composites from each of the 5-d menus for both diets were analyzed for macronutrients. The micronutrient content of the diets was calculated using USDA Handbook 8 (28); all nutrients were at or above the recommended dietary allowances and were not different between the two diets. Diets contained about one recommended dietary allowance of vitamin E from natural foods (calculated using values from USDA Handbook 8) and were supplemented with an additional 20 mg/d of d-α-tocopherol acetate (Bronson Pharmaceutical, St. Louis, MO).

TABLE 1
Nutrient Composition of Experimental Diets[a]

	Energy %	
Nutrient	DHA diet	Control diet
Protein	15.2 ± 1.3	14.6 ± 1.2
Carbohydrate	53.9 ± 6.0	56.4 ± 6.8
Fat, total	30.9 ± 4.3	29.0 ± 3.8
Saturated	8.9 ± 0.9	8.1 ± 0.9
Monounsaturated	9.2 ± 0.7	9.2 ± 0.8
n-6 Polyunsaturated	6.5 ± 0.8	8.5 ± 0.7
n-3 Polyunsaturated	2.8 ± 0.1	1.1 ± 0.1
Trans	1.9 ± 0.3	2.1 ± 0.3
P/S ratio	1.2	1.1
Cholesterol (mg/d)	360	360

[a]Data shown are the mean ± SD of five dietary composites from five different menu days. Polyunsaturated/saturated (P/S) ratio and cholesterol levels are calculated and not analyzed; hence, SD is not shown for these variables. DHA, docosahexaenoic acid.

Laboratory procedures. Blood samples were collected between 7: 00 and 8:00 A.M. after an overnight fast on study days 22, 30, 78, 85, 106, and 113, by antecubital venipuncture into evacuated tubes containing heparin for cell culture experiments, EDTA for blood cell count and phenotypic analysis, and no anticoagulant for preparation of sera.

Blood cell count and lymphocyte phenotypic analysis. For each blood draw, a complete and differential cell count was performed by using a Serono Baker Automated system (model 9000 diff; Allentown, PA). Phenotypic analysis for B (CD19+), T (CD3+), helper (CD3+, CD4+), suppressor (CD3+, CD8+), natural killer (CD3-,CD16+, 56+), and cytotoxic T (CTL; CD3+, CD16+, 56+) cells was done by using Becton-Dickinson FACStar flow cytometer (San Jose, CA) as previously reported (5). The numbers of lymphocytes producing IL2 were also determined with the flow cytometer, using instructions and reagents provided by Becton-Dickinson. The lymphocytes were activated by mixing 500 μL of whole blood with 500 μL RPMI-1640, containing 10 μg brefeldin A, 25 ng phorbol 12-myristate 13-acetate, and 1 μg ionomycin. An unstimulated tube containing only blood and brefeldin was used as a control. The tubes were incubated at 37°C in 5% CO_2 for 4 h. The T-cell surface antigen (CD3+) was stained for 30 min with R-phycoerythrin-Cyanin5-CD3 monoclonal antibody. The cells were incubated for 10 min in Facs Lysing Solution to lyse the red cells. The cells were then centrifuged and resuspended in Facs Permeabilizing Solution. After a 10-min incubation period, 2 mL of phosphate buffered saline was added, the cells centrifuged, and the supernatant discarded. The pellet was stained with fluorescent-conjugated intracellular IL2 monoclonal antibody and incubated for 30 min. The cells were washed once with phosphate buffered saline and resuspended in 1% paraformaldehyde. The number of IL2-producing cells was analyzed with a flow cytometer with gating on the CD3+ cells.

Proliferation of peripheral blood mononuclear cells (PBMNC). PBMNC were isolated using Histopaque-1077 as previously reported (5,6) and cultured in RPMI-1640 containing 10% autologous serum. One hundred microliters of the culture medium containing 1×10^5 PBMNC were inoculated

in each well of a 96-well flat-bottom culture plate. An additional 100 μL of the culture medium with or without the mitogens was added to each well. The mitogens used were phytohemagglutinin (PHA) and concanavalin A (Con A). Both PHA and Con A were used at three concentrations; final concentrations (mg/L) in the culture media were 2.5, 5, and 10. PBMNC were cultured for a total of 72 h; [^{3}H] thymidine, 37 K becquerel (Bq), in 50 μL, was added to each well during the last 24 h. PBMNC were collected on filter strips, and the radioactivity was determined using a Packard β-gas counter. [^{3}H] thymidine incorporation into cellular DNA (Bq/1000 cells) was used as the index of PBMNC proliferation.

Delayed type hypersensitivity (DTH) skin response and the serum IgG, C3, and IL2R. DTH response to seven recall antigens was assayed by intradermally injecting 0.1 mL of each antigen solution into the forearm. The antigens used were tuberculin purified-protein derivative (1 international test unit), mumps (4 complement-fixing test units), tetanus toxoid (1:100, vol/vol dilution of a solution containing 4 flocculation units/0.5 mL), candida (1:100, vol/vol dilution), trichophyton (1:30, vol/vol dilution), streptokinase streptase (100 KU/L), and coccidioidin (bioequivalent to U.S. reference coccidioidin 1:100, provided by the Office of Biologics, Food and Drug Administration, Washington, DC). The antigens were diluted with a diluent containing, per liter, 3 mL normal human serum and 9 g sodium chloride. Tuberculin purified-protein derivative, mumps, and tetanus toxoid were supplied by Connaught Laboratories Inc. (Swiftwater, PA). Candida (Dermatophyton O), trichophyton, and the antigen diluent were obtained from Hollister Stier (Spokane, WA). Streptokinase streptase and coccidioidin were purchased from Behringwereke A.G. (Marburg/Lahn, Germany, and Berkeley Biologicals (Berkeley, CA), respectively. Response to these antigens was determined by measuring mean induration diameters (mm) at 48 h after injections. Induration diameters with less than 4 mm were scored negative. Data are reported as the mean sum of induration diameters for all positive responses (induration score) and the number of positive responses to the seven antigens (antigen score).

Serum IgG and C3 concentrations were determined using a nephelometer as previously reported (6). Serum IL2R was quantified by using an ELISA kit purchased from Immunotech (Westbrook, ME).

Data analysis. For both dietary groups, two determinations of IR were made at the end of stabilization, middle, and end of the intervention periods (d 23, 30, 78, 85, 106, and 113). Means of the two measurements are shown in the Results section. A repeated measure of analysis of variance model was used to determine the effects of DHA on the indices of IR tested. A univariate, split-plot approach was taken using SAS PROC MIXED (29). Contrasts were constructed for comparisons among the stabilization and intervention periods, and in cases for which responses were linear, the control and DHA group slopes were compared. Changes in the parameters examined are considered significant for $P < 0.05$ unless otherwise stated.

RESULTS

The mean ± SEM for age (yr), weight (kg), and body mass index (kg/m^2) for subjects in DHA group ($n = 7$) and control group ($n = 4$) were 33.1 ± 1.8, 78.6 ± 4.2, 23.7 ± 0.8, and 33.3 ± 3.1, 74.7 ± 4.2, 23.1 ± 1.1, respectively. None of these physical characteristics was different between the two groups.

The average daily energy intake for all subjects was 11.7 MJ (2800 Kcal), and was not different between the two groups. The total fat intake was also similar between the two groups. Fatty acid composition of the two diets is shown in Table 2. Fatty acids other than DHA, linoleate, laurate, and myristate were not different between the two diets. The DHA diet contained 6.5 wt% of DHA, while the control diet contained less than 0.1% of it. Since DHA was incorporated by replacing equivalent amount of LA, the control diet contained 6.7% more LA than the DHA diet. The laurate and myristate contents of the DHA diet were approximately twice their levels in the basal diet, but they were still only 1.3 and 4.3% of the total fatty acids, respectively.

Effect of DHA on circulating white blood cells (WBC). The number of total circulating WBC, along with their differentiation into granulocytes or polymorphonuclear leukocytes (PMN), monocytes, lymphocytes, and various subsets of lymphocytes is shown in Table 3. This table shows that in the DHA group the number of circulating total WBC decreased by 10%, from the end of stabilization period (day 30) to the end of the intervention period (day 113). This decrease in WBC numbers was statistically significant ($P = 0.0001$). The decrease in WBC in the DHA group was primarily due to a decrease in the number of PMN, which were reduced by 21% ($P = 0.0001$). The decrease in the number of circulating PMN

TABLE 2
Fatty Acid Composition (wt%) of Experimental Diets[a]

FAME	DHA diet	Control diet
12:0 (laurate)	1.3 ± 0.1	0.7 ± 0.1*
14:0 (myristate)	4.3 ± 0.3	2.2 ± 0.4*
16:0 (palmitate)	16.6 ± 0.7	16.3 ± 0.7
16:1n-9	0.7 ± 0.2	0.9 ± 0.2
18:0 (stearate)	7.1 ± 0.5	7.5 ± 0.4
18:1*t*, all isomers	6.2 ± 0.5	7.0 ± 0.5
18:1n-9 (oleate)	26.6 ± 0.8	26.0 ± 0.7
18:1n-7	1.7 ± 0.1	2.0 ± 0.1
18:1n-5	1.5 ± 0.2	2.1 ± 0.4
18:2*tt* & 19:0	0.5 ± 0.1	0.6 ± 0.1
18:2n-6 (linoleate)	21.6 ± 1.2	28.3 ± 1.0*
18:3n-3 (linolenate)	2.6 ± 0.2	3.2 ± 0.1
22:0 (behenate)	0.2 ± 0.0	0.2 ± 0.0
20:5n-3 (eicosapentaenoate)	0.4 ± 0.1	0.3 ± 0.1
22:6n-5 (docosahexaenoate)	6.5 ± 0.22	>0.1 ± 0.1*
Total	98.0 ± 0.4	97.2 ± 0.3
Unknowns	2.0 ± 0.2	2.8 ± 0.1

[a]Data shown are mean ± SEM ($n = 5$). *Significantly different between two diets ($P < 0.05$). FAME, fatty acid methyl esters. See Table 1 for other abbreviation.

TABLE 3
Effect of DHA Feeding on Circulating White Blood Cells[a]

	DHA group (n = 7)		Control group (n = 4)	
Cell type	Study day 30	Study day 113	Study day 30	Study day 113
WBC[1]	5.11 ± 0.52	4.62 ± 0.44*	5.60 ± 0.51	5.53 ± 0.77
PMN[1]	2.81 ± 0.38	2.21 ± 0.28*	3.01 ± 0.44	3.08 ± 0.61
% of WBC	53.70 ± .50	47.50 ± .10*	52.35 ± 4.25	53.90 ± 3.90
Monocytes[1]	0.36 ± 0.04	0.31 ± 0.04	0.45 ± 0.04	0.46 ± 0.07
% of WBC	6.90 ± 0.71	6.44 ± 0.59	7.80 ± 0.70	7.91 ± 0.73
Lymphocytes[1]	2.00 ± 0.18	2.12 ± 0.21	2.17 ± 0.17	2.05 ± 0.15
% of WBC	39.50 ± 2.89	46.20 ± 2.85*	39.87 ± 3.91	38.15 ± 3.52
B (CD19+)[1]	0.20 ± 0.05	0.22 ± 0.05	0.22 ± 0.03	0.20 ± 0.03
T (CD3+)[1]	1.45 ± 0.14	1.59 ± 0.16	1.79 ± 0.26	1.63 ± 0.18
% producing IL2	39.15 ± 3.86	37.30 ± 1.93	44.95 ± 5.95	43.75 ± 3.45
Helper (CD3+, 4+)[1]	0.89 ± 0.08	0.94 ± 0.09	1.07 ± 0.15	0.91 ± 0.07
Suppressor (CD3+, 8+)[1]	0.55 ± 0.06	0.58 ± 0.08	0.64 ± 0.06	0.61 ± 0.07
NK (CD3-, 16+, 56+)[1]	0.25 ± 0.04	0.24 ± 0.04	0.25 ± 0.13	0.20 ± 0.07
CTL (CD3+,16+, 56+)[1]	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01

[a]WBC, white blood cells; PMN, polymorphonuclear leukocytes; CD, cluster designation; NK, natural killers; and CTL, cytotoxic T. Data shown are mean ± SEM for the number of subjects shown. Study day 30 and 113 correspond to the end of stabilization and intervention dietary periods, respectively. Although data are shown only for the end of the stabilization and intervention periods, data from study days 22,30,78,85,106, and 113 were all used to determine dietary effects using repeated measures of analysis of variance (ANOVA). Superscript(*) indicates significant change with DHA feeding (P = 0.0001), and superscript 1 indicates cell number × 10^{-9}/L blood. NK, See Table 1 for other abbreviations.

was also evident if these cells were expressed as percentage of the total WBC. The temporal changes in the number of circulating WBC and PMN for both the control and DHA groups are shown in Figure 1. This figure shows that the reduction in WBC and PMN numbers in the DHA group was evident within 50 d of DHA supplementation.

Table 3 also shows that the number of monocytes in the DHA group also decreased; however, it did not reach statistical significance. The number of total WBC, PMN, and monocytes in the control group, and that of lymphocytes in both groups remained unchanged throughout the study. The percentage of lymphocytes in the DHA group was increased (P = 0.0001) with DHA supplementation, because of the reduction in granulocytes in this group. DHA supplementation did not affect the number of B, total T, helper T, suppressor T, helper/suppressor ratio, CTL, or natural killer cells in circulation. The number of T cells producing IL2 was about 40% in both groups, and was not changed by DHA supplementation.

Effect of DHA feeding on PBMNC proliferation. Table 4 contains data regarding the proliferation of PBMNC cultured with three different concentrations of PHA and Con A. At the suboptimal concentration of PHA (2.5 mg/L), proliferation in both groups was higher at day 113, compared to the corresponding values at day 30. However this increase was not statistically significant and was not due to DHA intake. At this PHA concentration, PBMNC proliferation values at study day 78 for the DHA and control groups were 10.7 ± 1.4, and 9.2 ± 1.6, respectively, and were not different from the corresponding values at study day 30 (Table 4). PBMNC proliferation did not change at PHA concentration of 5 or 10 mg/L, or at any of the concentrations of Con A, throughout the study.

Effect of DHA on DTH skin response and serum concentrations of IgG, C3, and IL2R. Table 5 contains the data for both the groups regarding the immune indices listed above. Both the induration and antigen scores on day 113 were lower than at day 30 in both groups; however, none of these de-

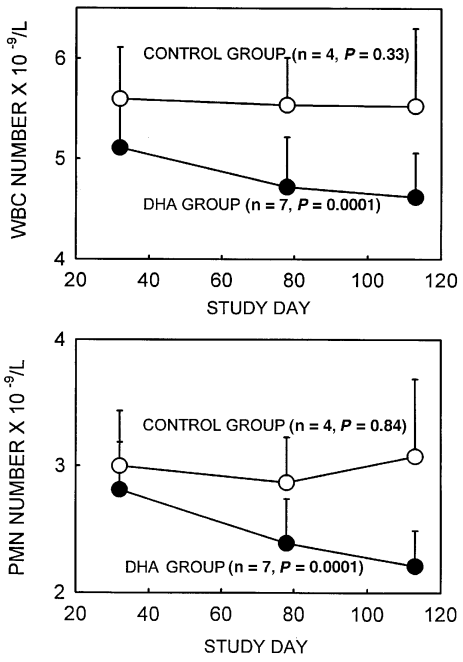

FIG. 1. Docosahexaenoic acid (DHA) feeding decreases the number of circulating white blood cells (WBC) and polymorphonuclear leukocytes (PMN). Data shown are the mean ± SEM for the number of subjects shown. The P values shown were obtained by analysis of variance.

TABLE 4
DHA Feeding Does Not Alter the Proliferation (Bq/1,000 cells) of PBMNC Cultured with Mitogens[a]

	DHA group (n = 7)		Control group (n = 4)	
Mitogen (mg/L)	Study day 30	Study day 113	Study day 30	Study day 113
PHA, 2.5	10.20 ± 0.97	13.61 ± 1.09	8.80 ± 1.00	12.56 ± 0.98
PHA, 5	13.65 ± 0.82	14.26 ± 0.83	13.37 ± 0.70	14.29 ± 1.11
PHA, 10	13.90 ± 0.92	14.47 ± 0.74	13.63 ± 0.75	15.20 ± 0.74
Con A, 2.5	6.24 ± 0.35	6.13 ± 0.45	5.55 ± 1.02	5.36 ± 0.76
Con A, 5	8.70 ± 0.43	8.52 ± 0.47	7.72 ± 0.89	7.95 ± 0.67
Con A, 10	10.08 ± 0.48	9.43 ± 0.46	9.04 ± 0.76	8.66 ± 0.95

[a]Data shown are mean ± SEM for the number of subjects shown for each group. Study days 30 and 113 correspond to the end of stabilization and intervention dietary periods, respectively. PHA, phytohemagglutinin; Con A, concanavalin A; Bq, becquerel; and PBMNC, peripheral blood mononuclear cells. None of these variables was altered by DHA supplementation as determined by repeated measures of ANOVA using data from study days 22, 30, 78, 85, 106, and 133. See Tables 1 and 3 for other abbreviations.

creases was statistically significant, and could not be attributed to DHA feeding. These changes were primarily due to a reduction in response to tetanus antigen. At day 30, six of the subjects in the DHA group tested positive for this antigen, with an induration (mean ± SEM) of 12.7 ± 2.4 mm. However, at day 113, only three of the subjects tested positive for this antigen in the DHA group, with an induration of 9.5 ± 0.5 mm. In the control group, all four subjects tested positive for tetanus on day 30, with an induration of 12.2 ± 2.4 mm. Only three subjects in the control group tested positive on day 113, with an induration of 8.0 ± 1.5 mm. It is possible that dietary enrichment or enrichment with DHA reduced the induration due to tetanus; however, because not all subjects responded to this antigen, we did not have adequate power to test the significance of the reduction in this response. Serum levels of IgG, C3, and IL2R were not affected by either diet (Table 5).

DISCUSSION

We examined if supplementation of diets with DHA triacylglycerol would lower human IR, as previously reported with fish oil supplementation. In contrast to most of the previous studies with fish oils, we maintained total fat intake constant, supplemented DHA in the absence of dietary EPA, and ensured adequate vitamin E intake by additional supplementation of d-α-tocopherol acetate at 20 mg/d. All these factors are important in evaluating the effects of n-3 PUFA on human IR.

Dietary DHA enrichment did not lower lymphocyte proliferation or the DTH skin response in the present study unlike results reported in a number of human studies in which fish oils or purified EPA were added to the diets without adjustment for the extra fat intake (8–14). There was no change in the number of lymphocytes producing IL2 or in the ratios between helper and suppressor T cells, as reported by others using EPA (8,13). Neither did DHA feeding alter the serum levels of IgG, C3, and IL2R. We believe the low and constant fat content of our experimental diets is the most likely reason why DHA did not inhibit the indices of IR as previously found by others with fish oils or EPA. It is likely that in studies with fish oils the increase in both total fat and n-3 PUFA contributed to the inhibition of IR.

Fish oils also contain EPA, which inhibits IR in humans (13,14,19), and it may be a more potent inhibitor than DHA. About 9% of the dietary DHA is retroconverted to EPA in humans (30,31). DHA supplementation raised its concentration from 1.8 to 8.1 wt% of plasma fatty acids, and that of plasma EPA from 0.38 to 3.39 wt% in the subjects included in our current study (32). Whether this amount of EPA is adequate to inhibit IR, or it is metabolized differently from the dietary

TABLE 5
DHA Feeding Does Not Alter DTH Response, and Serum Concentrations of IgG, C3, IL2R[a]

	DHA group (n = 7)		Control group (n = 4)	
	Study day30	Study day 113	Study day 30	Study day 113
Induration (mm)	39.4 ± 4.6	33.4 ± 6.4	35.1 ± 4.2	23.8 ± 1.3
Antigen Score	3.2 ± 0.4	3.0 ± 0.4	3.0 ± 0.4	2.5 ± 0.6
IgG (μmol)	86.0 ± 8.4	91.6 ± 8.4	85.1 ± 3.7	93.0 ± 8.2
C3 (μmol)	9.9 ± 0.4	9.8 ± 0.4	11.4 ± 1.5	11.8 ± 0.9
IL2R (nmol)	188.0 ± 17.1	189.3 ± 17.5	170.4 ± 15.3	195.1 ± 45.6

[a]DTH, delayed type hypersensitivity skin response; IgG, immunoglobulin G; C3, complement fraction 3; IL2R, interleukin 2 receptor. Data shown are mean ± SEM for the number of subjects shown for each group. Study days 30 and 113 correspond to the end of stabilization and intervention dietary periods, respectively. Antigen score refers to the number of antigens tested positive at 48 h after the application of 7 recall antigens. None of the variables was altered by DHA supplementation. See Table 1 for other abbreviation.

EPA is unknown. With the exception of a decrease in arachidonic acid, the plasma concentration of other fatty acids was not changed with DHA feeding (32). The modest increase in vitamin E intake in our study may also have prevented the inhibition of IR by DHA, as others (15) have shown that very high doses of α-tocopherol (200 mg/d) can overcome the inhibition of IR caused by fish oils (15 g/d). The amount of DHA in our study is comparable to the total of EPA and DHA provided from 15 g of fish oil; however, we provided only 10% of the vitamin E provided by these workers.

Which of the three factors (total fat, EPA, vitamin E) discussed above is more important cannot be answered from our study design. Regardless of the mechanisms involved, our results show that if total fat intake is maintained constant, a moderately high intake of DHA as triacylglycerol does not inhibit several indices of human IR that have previously been reported to be inhibited by fish oils, purified EPA, or flaxseed oil.

Our results do show a significant decrease in the number of circulating PMN in the subjects fed the DHA-containing diet. We are aware of only one human study with n-3 fatty acids where such effects have been reported in abstract form (33). Results from our current study regarding the number of circulating PMN contrast our recent findings with arachidonic acid feeding (5), where we found significant increase in their number in peripheral blood in the group fed arachidonic acid-containing diet. These opposing effects of DHA and arachidonic acid on the number of circulating PMN are consistent with their many other opposing effects on several physiological functions. The concept of dietary fats affecting the formation or maturation of WBC is relatively new and poorly understood. However, there are reports showing the inhibition of tumor development in animals fed diets supplemented with n-3 fatty acids (34,35). Results from a recent study by other investigators (36) showed that the number of colony-forming units–granulocyte macrophage in the bone marrow increased twofold in rats fed diets containing DHA, compared to the corresponding numbers in the rats fed a control or n-6 PUFA diet. If all of these colony-forming units attained maturation and their release into blood was proportional to their numbers in the marrow, then the number of PMN and monocytes should be higher in the blood of DHA-fed rats than those in the control or n-6 PUFA diet-fed rats. Unfortunately, these workers did not perform blood cell counts. The decrease in the number of PMN in drawn blood as seen in our study and the increase in the colony-forming units in the bone marrow of rats seem inconsistent; however, these two results become consistent if DHA blocks the migration of these cells from bone marrow to blood or increases their margination in the circulation. Of course, the differences could be simply due to species differences, but still it is important that future studies in this area monitor both colony-forming units in bone marrow and the number of granulocytes and monocytes in blood.

Colony-forming units are the precursors of both monocytes and PMN, yet we found significant decrease in the number of circulating PMN only. A close look at our data in Table 3 suggests that the number of circulating monocytes also decreased in the subjects fed the DHA-containing diet, but this decrease did not attain statistical significance. We may not be able to detect the change in the number of circulating monocytes, because of their relatively small numbers compared with PMN numbers. Results from the rat study suggest that this is the most likely explanation for our results with PMN and monocytes. However, other explanations such as the effect of DHA on PMN proliferative and storage pools, their apoptosis and egress from bone marrow could also explain our results. The number of lymphocytes, which are derived from a different progenitor cell than the PMN and monocytes, was not affected by DHA feeding. Actually, lymphocytes as a percentage of WBC increased because of the decrease in PMN count. Thus, it seems that DHA effect was specific for granulocytes or for granulocyte-monocytes.

The number of circulating PMN is influenced by a number of hormones, growth factors, toxins, and cytokines. We are not sure of the mechanisms by which DHA caused the decrease in the number of circulating PMN. We did monitor the concentrations of cortisol, T3, and T4 in the sera of our study subjects (not shown); none of these hormones changed in both groups during the study. Feeding diets containing fish oil to healthy subjects decreased leukotriene B_4 (LTB_4) production by isolated neutrophils and monocytes (12), and LTB_4 is known to enhance neutrophil chemotaxis and diapedesis (37). It is possible that increased intake of DHA reduced LTB_4 production in our study, which may have mediated the decrease in the number of circulating PMN.

It is possible that some of the effects observed in this study were due to a reduction in LA intake rather than the increase in DHA intake. The intake of LA was decreased by about 7 g/d in the high DHA diet compared to the basal diet. The subjects on the high DHA diet were still receiving more than 20 g/d LA, an amount that will flood the fatty acid metabolic acid pathways and is well above any known requirements for LA. The plasma LA content in the basal and high DHA diets fed subjects was 39 and 36%, respectively. The difference between the two groups was not statistically significant. The basal diet contained less than 50 mg DHA per day, and the high DHA diet had 6 g/d. Thus, it is unlikely that any of the changes in immune functions were due to a 20% reduction in LA intake. These were most likely the result of increase in DHA intake.

Although the PMN counts decreased by 21%, the residual counts were still within the clinically normal ranges. The subjects fed the high DHA diet did not show an increase in the rate of infections or any other health problems. The risk for infections may increase, if the subjects had a marginal PMN count prior to DHA supplementation, or if a high amount of DHA was consumed for an extended period of time. DHA may also inhibit other aspects of human IR, if it is added to diets high in total fat or low in n-6 PUFA and/or antioxidant nutrients. These questions need to be addressed in future studies. Until more information is available, people supplementing their diets with DHA should have a periodic evaluation of their immune status.

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